

# THE RÔLE OF SALTS IN THE PRESERVATION OF LIFE

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# REMOTE STORAGE

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## THE RÔLE OF SALTS IN THE PRESERVATION OF LIFE

### I

LESS is known of the rôle of the salts in the animal body than of the rôle of the three other main food-stuffs, namely, carbohydrates, fats and proteins. As far as the latter are concerned, we know at least that through oxidation they are capable of furnishing heat and other forms of energy. The neutral salts, however, are not oxidizable. Yet it seems to be a fact that no animal can live on an ash-free diet for any length of time, although no one can say why this should be so. We have a point of attack for the investigation of the rôle of the salts in the fact that the cells of our body live longest in a liquid which contains the three salts,  $\text{NaCl}$ ,  $\text{KCl}$  and  $\text{CaCl}_2$  in a definite proportion, namely, 100 molecules  $\text{NaCl}$ , 2.2 molecules  $\text{KCl}$  and 1.5 molecules of  $\text{CaCl}_2$ . This proportion is identical with the proportion in which these salts are contained in sea-water; but the concentration of the three salts is not the same in both cases. It is about three times as high in the sea-water as in our blood serum.

Biologists have long been aware of the fact that the ocean has an incomparably richer fauna than fresh-water lakes or streams and it is often assumed that life on our planet originated in the ocean. The fact that the salts of Na, Ca and K exist

in the same proportion in our blood serum as in the ocean has led some authors to the conclusion that our ancestors were marine animals, and that, as a kind of inheritance, we still carry diluted sea-water in our blood. Statements of this kind have mainly a metaphorical value, but they serve to emphasize the two facts, that the three salts,  $\text{NaCl}$ ,  $\text{KCl}$  and  $\text{CaCl}_2$ , exist in our blood in the same relative proportion as in the ocean and that they seem to play an important rôle in the maintenance of life.

I intend to put before you a series of experiments which seem to throw some light on the mechanism by which the solutions surrounding living cells influence their duration of life.

### II

In order to give a picture of the extent to which the life of many animals depends upon the cooperation of the three salts I may mention experiments made on a small marine crustacean, *Gammarus*, of the Bay of San Francisco. If these animals are suddenly thrown into distilled water, their respiration stops (at a temperature of 20° C.) in about half an hour. If they are put back immediately after the cessation of respiration into sea-water, they can recuperate. If ten minutes or more are allowed to elapse before bringing them back into the sea-water, no recuperation is possible. Since in this case death is caused

<sup>1</sup> Carpenter lecture delivered at the Academy of Medicine of New York, October 19, 1911.

obviously through the entrance of distilled water into the tissues of the animals, one would expect that the deadly effect of distilled water would be inhibited if enough cane sugar were added to the distilled water to make the osmotic pressure of the solution equal to that of the sea-water. If, however, the animals are put into cane-sugar solution, the osmotic pressure of which is equal to that of sea-water, the animals die just about as rapidly as in distilled water. The same is true if the osmotic pressure of the sugar solution is higher or lower than that of the sea-water. The sugar solution is, therefore, about as toxic for the animals as the distilled water, although in the latter case water enters into the tissues of the animal, while in the former case it does not.

If the sea-water is diluted with an equal quantity of distilled water in one case, and of isotonic cane-sugar solution in the other case, in both cases the duration of life is shortened by practically the same amount.

If the crustaceans are brought into a pure solution of  $\text{NaCl}$ , of the same osmotic pressure as the sea-water, they also die in about half an hour. If to this solution a little calcium chloride be added in the proportion in which it is contained in the sea-water the animals die as rapidly as without it. If, however, both  $\text{CaCl}_2$  and  $\text{KCl}$  are added to the sodium chloride solution, the animals can live for several days. The addition of  $\text{KCl}$  alone to the  $\text{NaCl}$  prolongs their life but little.

If  $\text{KCl}$  and  $\text{CaCl}_2$  are added to a cane sugar solution isotonic with sea-water, the animals die as quickly or more so than in the pure cane-sugar solution.

If other salts be substituted for the three salts the animals die. The only substitution possible is that of  $\text{SrCl}_2$  for  $\text{CaCl}_2$ . We find also that the proportion in which the three salts of sodium, calcium and

potassium have to exist in the solution can not be altered to any extent. All this leads us to the conclusion, that in order to preserve the life of the crustacean *Gammarus*, the solution must not only have a definite concentration or osmotic pressure but that this osmotic pressure must be furnished by definite salts, namely, sodium chloride, calcium chloride and potassium chloride in the proportion in which these three salts exist in the sea-water (and in the blood); this fact could also be demonstrated for many other marine animals. The relative tolerance of various cells and animals for abnormal salt solutions is, however, not the same, a point which we shall discuss later on.

### III

What is the rôle of the salts in these cases? The botanists have always considered salt solutions as nutritive solutions. It is a well-known fact that plants require definite salts, *e. g.*, nitrates and potassium salts, for their nutrition, and the question now arises whether the three salts  $\text{NaCl}$ ,  $\text{KCl}$  and  $\text{CaCl}_2$ , which are needed for the preservation of animal life, play the rôle of nutritive salts. Experiments which I made on a small marine fish, *Fundulus*, proved beyond question that this is not the case. If the young, newly hatched fish are put into a pure solution of sodium chloride of the concentration in which this salt is contained in sea-water, the animals very soon die. If, however,  $\text{KCl}$  and  $\text{CaCl}_2$  be added to the solution in the right proportion, the animals can live indefinitely. These fish, therefore, behave in this respect like *Gammarus* and the tissues of the higher animals, but they differ from *Gammarus* and the majority of marine animals inasmuch as the fish can live long, and in some cases, indefinitely, in distilled and fresh water, and certainly in a very dilute solution of sodium chloride. From this fact I drew

the conclusion that KCl and CaCl<sub>2</sub> do not act as nutritive substances for these animals, that they only serve to render NaCl harmless if the concentration of the latter salt is too high. I succeeded in showing that as long as the sodium-chloride solution is very dilute and does not exceed the concentration of m./8, the addition of KCl and CaCl<sub>2</sub> is not required. Only when the solution of NaCl has a concentration above m./8 does it become harmful and does it require the addition of KCl and CaCl<sub>2</sub>.

The experiments on *Fundulus*, therefore, prove that a mixture of NaCl + KCl + CaCl<sub>2</sub> does not act as a nutritive solution, but as a *protective* solution. KCl and CaCl<sub>2</sub> are only necessary in order to prevent the harmful effects which NaCl produces if it is alone in solution and if its concentration is too high. We are dealing, in other words, with a case of antagonistic salt action; an antagonism between NaCl on the one hand and KCl and CaCl<sub>2</sub> on the other. The discovery of antagonistic salt action was made by Ringer, who found that there is a certain antagonism between K and Ca in the action of the heart. When he put the heart of a frog into a mixture of NaCl + KCl he found that the contractions of the heart were not normal, but they were rendered normal by the addition of a little CaCl<sub>2</sub>. A mixture of NaCl + CaCl<sub>2</sub> also caused abnormal contractions of the heart, but these were rendered normal by the addition of KCl. Ringer drew the conclusion that there existed an antagonism between potassium and calcium, similar to that which Schmiedeberg had found between different heart poisons, *e. g.*, atropin and muscarin. Biedermann had found that alkaline salt solutions cause twitchings in the muscle and Ringer found that the addition of Ca inhibited these twitchings. Since these experiments were made many examples of the antagonistic action of salts have become known.

It had generally been assumed that the antagonistic action of two salts was based on the fact that each salt, when applied singly, acted in the opposite way from that of its antagonist. We shall see that in certain cases of antagonistic salt action at least this view is not supported by fact.

## IV

What is the mechanism of antagonistic salt action? I believe that an answer to this question lies in the following observations on the *eggs* of *Fundulus*. If these eggs are put immediately after fertilization into a pure sodium chloride solution which is isotonic with sea-water, they usually die without forming an embryo. If, however, only a trace of a calcium salt, or of any other salt with a bivalent metal (with the exception of Hg, Cu or Ag) is added to the m./2 NaCl solution, the toxicity of the solution is diminished or even abolished. Even salts which are very poisonous, namely, salts of Ba, Zn, Pb, Ko, Ni, Mn and other bivalent metals, are able to render the pure solution of sodium chloride harmless, at least to the extent that the eggs can live long enough to form an embryo. The fact that a substance as poisonous as Zn or lead can render harmless a substance as indifferent as sodium chloride seemed so paradoxical that it demanded an explanation, and this explanation casts light on the nature of the protective or antagonistic action of salts. For the antagonistic action of a salt of lead or zinc against the toxic action of sodium chloride can only consist in the lead salt protecting the embryo against the toxic action of the NaCl. But how is this protective action possible?

We have mentioned that if we put the young fish, immediately after hatching, into a pure m./2 solution of sodium chloride the animals die very quickly, but that they live indefinitely in the sodium chloride solution if we add both CaCl<sub>2</sub> and KCl. How

does it happen that for the embryo, *as long as it is in the egg shell*, the addition of  $\text{CaCl}_2$  to the  $\text{NaCl}$  solution suffices, while if the fish is *out of the shell* the addition of  $\text{CaCl}_2$  alone is no longer sufficient and the addition of  $\text{KCl}$  also becomes necessary? Moreover, if we try to preserve the life of the fish *after it is taken out of the egg* in an m./2 sodium chloride solution by adding  $\text{ZnSO}_4$ , or lead acetate, to the solution we find that the fish die even much more quickly than without the addition.

If we look for the cause of this difference our attention is called to the fact that the fish, as long as it is in the egg, is separated from the surrounding solution by the egg membrane. This egg membrane possesses a small opening, the so-called micropyle, through which the spermatozoon enters into the egg. I have gained the impression that this micropyle is not closed as tightly immediately after fertilization as later on, since the *newly fertilized* egg is killed more rapidly by an m./2 solution of  $\text{NaCl}$  than it is killed by the same solution one or two days after fertilization. One can imagine that the micropyle contains a wad of a colloidal substance which is hardened gradually to a leathery consistency if the egg remains in the sea-water. With the process of hardening, or tanning, it becomes more impermeable for the  $\text{NaCl}$  solution. This process of hardening is brought about apparently very rapidly if we add to the m./2  $\text{NaCl}$  solution a trace of a salt of a bivalent metal like Ca, Sr, Ba, Zn, Pb, Mn, Ko and Ni, etc. It is also possible that similar changes take place in the whole membrane. The process of rendering the m./2  $\text{Na}$  solution harmless for the embryo of the fish, therefore, depends apparently upon the fact that the addition of the bivalent metals renders the micropyle or perhaps the whole membrane of the egg more impermeable to  $\text{NaCl}$  than was the case before.

But these are only one part of the facts which throw a light upon the protective or antagonistic action of salts. Further data are furnished by experiments which I made together with Professor Gies, also on the eggs of *Fundulus*. Gies and I were able to show that not only are the bivalent metals able to render the sodium chloride solution harmless, but that the reverse is also the case, namely, that  $\text{NaCl}$  is required to render the solutions of many of the bivalent metals, for instance  $\text{ZnSO}_4$ , harmless. (That the  $\text{SO}_4$  ion has nothing to do with the result was shown before by experiments with  $\text{Na}_2\text{SO}_4$ .)

TABLE I

Nature of the Solution	Percentage of the Eggs Forming an Embryo
100 c.c. distilled water .....	49
100 c.c. distilled water +8 c.c. m./32 $\text{ZnSO}_4$	0
100 c.c. m./64 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	0
100 c.c. m./32 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	3
100 c.c. m./16 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	8
100 c.c. m./8 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	44
100 c.c. m./4 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	38
100 c.c. 3/8 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	37
100 c.c. m./2 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	34
100 c.c. 5/8 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	29
100 c.c. 6/8 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	8
100 c.c. 7/8 $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	6
100 c.c. m. $\text{NaCl}$ +8 c.c. m./32 $\text{ZnSO}_4$	1

If the eggs of *Fundulus* are put immediately after fertilization into distilled water, a large percentage of the eggs develop, often as many as one hundred per cent., and the larvae and embryos formed in the distilled water are able to hatch. If we add, however, to 100 c.c. of distilled water that quantity of  $\text{ZnSO}_4$  which is required to render the  $\text{NaCl}$  solution harmless, all the eggs are killed rapidly and not a single one is able to form an embryo. If we add varying amounts of  $\text{NaCl}$  we find that, beginning with a certain concentration of  $\text{NaCl}$ , this salt inhibits the toxic effects of  $\text{ZnSO}_4$  and many eggs are able to form

an embryo. This can be illustrated by Table I.

This table shows that the addition of NaCl, if its concentration exceeds a certain limit, namely, m./8, is able to render the ZnSO<sub>4</sub> in the solution comparatively harmless.

If we now assume that ZnSO<sub>4</sub> renders the 5/8 m. NaCl solution harmless by rendering the egg membrane comparatively impermeable for NaCl we must also draw the opposite conclusion, namely, that NaCl renders the egg membrane comparatively impermeable for ZnSO<sub>4</sub>. We, therefore, arrive at a new conception of the mutual antagonism of two salts, namely, that this antagonism depends, in this case at least, upon a *common, cooperative* action of *both* salts on the egg membrane, by which action this membrane becomes completely or comparatively impermeable for *both* salts. And from this we must draw the further conclusion that the fact that each of these salts, if it is alone in the solution, is toxic, is due to its comparatively rapid diffusion through the membrane, so that it comes into direct contact with the protoplasm of the germ.

As long as we assumed that each of the two antagonistic salts acted, if applied singly, in the opposite way from its antagonist, it was impossible to understand these experiments or find an analogue for them in colloid chemistry. But if we realize that NaCl alone is toxic because it is not able to render the egg membrane impermeable; and that ZnSO<sub>4</sub> if alone in solution is toxic for the same reason; while both combined are harmless (since for the "tanning" of the membrane the action of the two salts is required) these experiments become clear.

We may, for the sake of completeness, still mention that salts alone have such antagonistic effects; glycerine, urea and alcohol have no such action. On the other

hand, ZnSO<sub>4</sub> was not only able to render NaCl harmless, but also LiCl, NH<sub>4</sub>Cl, CaCl<sub>2</sub> and others; and *vice versa*.

These experiments on the egg of *Fundulus* are theoretically of importance, since they leave no doubt that in this case at least the "antagonistic" action of salts consists in a modification of the egg membrane by a combined action of two salts, whereby the membrane becomes less permeable for both salts.

## V

It is not easy to find examples of experiments in the literature which are equally unequivocal in regard to the character of antagonistic salt action; but I think that some recent experiments by Osterhout satisfy this demand.

It has long been a question whether or not cells are at all permeable for salts. Nobody denies that salts diffuse much more slowly into the cells than water; but some authors, especially Overton and Hoeber, deny categorically that salts can diffuse at all into the cells. Overton's view is based partly on experiments on plasmolysis in the cells of plants. If the cells of plants, for example, those of *Spirogyra*, are put into a solution of NaCl or some other salt of sufficiently high osmotic pressure, the volume of the contents of the cell decreases through loss of water and the protoplasm retracts, especially from corners of the rigid cellulose walls. Overton maintains that this plasmolysis is permanent, and concludes from this that only water but no salt, can diffuse through the cell-wall; since otherwise salts should gradually diffuse from the solution into the cell, and through this increase in the osmotic pressure of the cell the water should finally diffuse back into the cell and restore the normal volume of the cell. According to Overton this does not happen.

Osterhout has recently shown that Over-

ton's observations were incomplete in a very essential point and that in reality the plasmolysis, which occurs in this case when the cell is put into the hypertonic solution, disappears again in a time which varies with the nature of the salt in solution. This stage of reversion of plasmolysis had been overlooked by Overton. If the cell, however, remains permanently in the hypertonic sodium chloride solution, afterwards again a shrinking of the contents of the cell takes place, which superficially resembles plasmolysis, but which in reality has nothing to do with plasmolysis, but is a phenomenon of death. That this second "false plasmolysis," as Osterhout calls it, has nothing to do with the hypertonic character of the solution was proved by the fact that hypotonic solutions of toxic substances may produce the same phenomenon.

In one experiment which Osterhout describes, "a portion of a *Spirogyra* filament was plasmolyzed in .2 m.  $\text{CaCl}_2$ , but not in .195 m.  $\text{CaCl}_2$ . A .29 m.  $\text{NaCl}$  solution has approximately the same osmotic pressure as a .2 m.  $\text{CaCl}_2$  solution. But on placing another portion of the same *Spirogyra* filament in a .29 m.  $\text{NaCl}$  solution the expected plasmolysis does not occur and it is impossible to plasmolyze the cells until they are placed in .4 m.  $\text{NaCl}$ ." Osterhout explains this difference in the concentration of the two salts required for plasmolysis by the assumption that  $\text{NaCl}$  diffuses more rapidly into the cell than  $\text{CaCl}_2$ , a conclusion which I reached also on the basis of my earlier experiments on animals.

Osterhout's experiments also show that the antagonism of  $\text{NaCl}$  and  $\text{CaCl}_2$  depends partly on the facts that the two salts inhibit each other from diffusing into the cells, and this conclusion is based among others upon the following experiment. "By dividing a *Spirogyra* filament into several portions it was found that it was plasmolyzed in .2 m.  $\text{CaCl}_2$  and in .38 m.

$\text{NaCl}$ , but neither in .195 m.  $\text{CaCl}_2$  nor in .375 m.  $\text{NaCl}$ . On mixing 100 c.c. .375 m.  $\text{NaCl}$  with 10 c.c. .195 m.  $\text{CaCl}_2$  and placing other portions of the same filament in it, prompt and very marked plasmolysis occurred."

The explanation for this observation lies in the fact that in the mixture of  $\text{NaCl}$  and  $\text{CaCl}_2$  the two salts render their diffusion into the cell mutually more difficult. After a longer period of time the plasmolyzed cells can expand again in a mixture of  $\text{NaCl}$  and  $\text{CaCl}_2$ , but that occurs much later than if they are in the pure  $\text{NaCl}$  solution.

These experiments are the analogue of the observation on the embryo of the eggs of *Fundulus* in which a pure solution of  $\text{ZnSO}_4$  diffused rapidly through the membrane or micropyle, while, if both salts were present, the diffusion was inhibited or considerably retarded.

While the observations of Osterhout show that Overton was not justified in using the experiments on plasmolysis to prove that the neutral salts can not diffuse into the cells, yet they do not prove that these salts diffuse into the cell under normal conditions. In Osterhout's experiments the cells are in strongly hypertonic solutions and it does not follow that such solutions act like isotonic, perfectly balanced solutions.

## VI

Wasteneys and I have recently shown that the toxic action of acids upon *Fundulus* can be annihilated by salts. If we add 0.5 c.c. *N/10* butyric acid to 100 c.c. of distilled water these fish die in  $2\frac{1}{2}$  hours or less. In solutions which contain 0.4 c.c. or less acid they can live for a week or more. If we add, however, 0.5 c.c. of butyric acid to 100 c.c. of solutions of  $\text{NaCl}$  of various concentration, we find that above a certain limit the  $\text{NaCl}$  can render the acid harmless. It is needless to say that the  $\text{NaCl}$  used in these experiments was strictly neu-

tral and that the amount of acid present in the mixture of acid and salt was measured. The following experiment may serve as an example. Each solution contained six fish at the beginning of the experiment.

TABLE II

After	Number of Surviving Fish in 0.5 c.c. N/10 Butyric Acid						
	+0	4.0	6.0	8.0	10.0	12.0	15.0
	c.c. m./2 NaCl in 100 c.c. of the Solution						
2 hours.....	0	0	0	2	3	3	6
4 hours .....				0	3	2	5
1 day.....					1	1	5
2 days.....					1	0	5
3 days.....					1		5
4 days.....					1		5

If the amount of acid was increased, the amount of NaCl also had to be increased to render the acid harmless. In order to render 0.5 c.c. N/10 butyric acid pro 100 c.c. solution harmless, 10 c.c. m./2 NaCl had to be added; while 0.8 c.c. butyric acid required 20 c.c. and 1.0 c.c. butyric acid required about 28 c.c. m./2 NaCl in 100 c.c. of the solution.

Not only butyric acid, but any kind of acid, could be rendered harmless by neutral salts, *e. g.*, HCl by NaCl.

It is of great importance that the antagonistic action of  $\text{CaCl}_2$  was found to be from 8 to 11 times as great or powerful as the action of NaCl. This harmonizes with the general observation that the protective action of  $\text{CaCl}_2$  for the life of cells is greater than that of any other substance.

Wasteneys and I could show that the rate of the absorption of acid by the fish is the same in solutions with and without salt. This proves that the action of the salts consisted in this case not in preventing the diffusion or absorption of the acid, but in modifying the deleterious effect of the absorbed acid.

We can state a little more definitely the cause of death by acid. If we put the fish into a weak acid solution in distilled water

just strong enough to kill the fish in from 1 to 2 hours (*e. g.*, 500 c.c.  $\text{H}_2\text{O} + 2.0$  c.c. N/10 HCl), we notice that the acid very soon makes the normally transparent epidermis of the fish opaque, and a little later the epidermis falls off in pieces and shreds. This, however, is probably not the direct cause of the death, but I am inclined to assume that the fish die from suffocation caused by a similar action of the acid upon the gills.

The action of the acid upon the epidermis of the body as well as upon the gills is prevented through the addition of neutral salts.

It is well known that the action of acids upon proteins can be inhibited by neutral salts. Thus the internal friction of certain protein solutions is increased by acids while the addition of neutral salts inhibits this effect (Pauli). The swelling of gelatine caused by acid is inhibited by salts (Procter).

It is possible that in the experiments with acid the fish is killed in the following way. The acid causes certain proteins in the surface layer of the epithelial cells of the gills and of the skin to swell, whereby this surface layer becomes more permeable for the acid. The acid can now diffuse into the epithelial cells and act on the protoplasm, whereby the cells are killed. If salts are present in the right concentration, the combined action of acid and salt causes a dehydration of the surface film of these cells, as it does in the experiments on gelatine or as in the cases of tanning of hides by the combined action of acids and salt solutions. This combined dehydrating or "tanning" action of acid and salts on the surface of the epithelial cells of the gills diminishes the permeability of this layer for the acids and prevents them from diffusing into the cells and thus destroying the protoplasm. In this way the gills are kept intact and the life of the fish is saved.

As long as the amount of acid is small the amount absorbed is not essentially diminished by the presence of salts; but while in the presence of salts the acid is consumed in the tanning action of the surface layer of the cells, or is absorbed in this layer; if no salt is present part of the acid diffuses into the epithelial cells and kills the latter.

### VII

We have thus far considered the cases of antagonism between two electrolytes only. The case of the antagonism between three electrolytes is a little more complicated.

We choose as an example the antagonism between NaCl, KCl and CaCl<sub>2</sub>—the antagonism which is most important in life phenomena. If the mechanism of the antagonism between NaCl, on the one hand, and KCl and CaCl<sub>2</sub>, on the other, is of the same nature as that between NaCl and ZnSO<sub>4</sub> in the case of the eggs of *Fundulus*, it must be possible to show that not only is NaCl toxic if it is alone in solution, and that it is rendered harmless by the two other salts, but that the reverse is true also. This can be proved in the case of KCl. To demonstrate it, we have again to experiment on organisms which are, in wide limits, independent of the osmotic pressure of the surrounding solution since the concentration of the KCl in sea-water is very low. The experiments were carried out by Mr. Wasteneys and myself on *Fundulus*. The method consisted in putting six fish, after washing them twice with distilled water, into 500 c.c. of the solution. It was ascertained from day to day how many fish survived.

When the fish were put into pure solutions of KCl of the concentration in which this salt is contained in the sea-water (2.2 c.c. m./2 KCl in 100 c.c. of the solution) they died mostly in less than two days. This is not due to the low concentration of the KCl solution, which is only 1/50

of that of the sea-water, since the fish can live indefinitely in a pure NaCl solution of the same concentration as that in which the KCl exists in the sea-water.

If we add to the toxic quantities of KCl increasing quantities of NaCl, we find that as soon as the solution contains 17 or more molecules of NaCl to one molecule of KCl, the toxic action of KCl is considerably diminished, if not completely counteracted. The following table may serve as an example.

TABLE III

After Days	Number of Surviving Fish in 2.2 c.c. m./2 KCl in 100 c.c.							
	H <sub>2</sub> O	m./100	m./20	m./8	m./4	3 m./8	m./2	NaCl
1	2	1	3	4	6	6	6	
2	0	0	0	0	6	5	6	
3					6	4	6	
4					5	3	5	
5					5	3	4	
6					5	3	1	
7					5	3	0	
14					4	3		

More accurate determinations showed that already a 3/16 m. NaCl solution renders the solution of 2.2 c.c. m./2 KCl in 100 c.c. of the solution harmless.

TABLE IV

	Coefficient of Antagonization
0.6 c.c. m./2 KCl rendered harmless in 100 c.c. 3/64 m. NaCl .....	1/16
0.7 c.c. m./2 KCl rendered harmless in 100 c.c. 4/64 m. NaCl .....	1/18
0.9 c.c. m./2 KCl rendered harmless in 100 c.c. 5/64 m. NaCl .....	1/17
1.0 c.c. m./2 KCl rendered harmless in 100 c.c. 5/64-6/64 m. NaCl ....	1/16-1/19
1.1 c.c. m./2 KCl rendered harmless in 100 c.c. 6/64 m. NaCl .....	1/17
1.65 c.c. m./2 KCl rendered harmless in 100 c.c. 5/32 m. NaCl .....	1/19
2.2 c.c. m./2 KCl rendered harmless in 100 c.c. 6/32 m. NaCl .....	1/17
2.75 c.c. m./2 KCl rendered harmless in 100 c.c. 7/32 m. NaCl .....	1/16
3.3 c.c. m./2 KCl rendered harmless in 100 c.c. 9/32 m. NaCl .....	1/17

It was next determined whether different concentrations of KCl required different

concentrations of NaCl. It was found that the coefficient of antagonization KCl/NaCl has an approximately constant value, namely, about 1/17, as Table IV. shows.

What happens if we vary this ratio? If we add too little NaCl to the KCl solution, namely, only 1 to 10 molecules NaCl to 1 molecule of KCl, the solution becomes more harmful than if KCl is alone in solution; if we add considerably more than 17 molecules NaCl, *e. g.*, 50 molecules to one molecule of KCl, the solution becomes toxic again; and the more so the higher the concentration of NaCl. This indicates that the antagonistic effect requires a rather definite ratio of the two salts. This furnishes the reason why an m./2 solution can, as a rule, not be rendered completely harmless by the mere addition of KCl, but that in addition CaCl<sub>2</sub> is needed.

If we add to 100 c.c. m./2 NaCl enough KCl to make the ratio KCl:NaCl = 1/17 we find that the antagonization of KCl:NaCl becomes incomplete. If the amount of KCl in 100 c.c. of the solution exceeds 2.2 c.c. m./2 KCl, antagonization is still to some extent possible, but it becomes more incomplete the higher the concentration of KCl. For this reason it is not possible to render an m./2 solution of NaCl harmless by the mere addition of KCl.

TABLE V

	Coefficient of Antagonization KCl/CaCl <sub>2</sub>
1.1 c.c. m./2 KCl in 100 c.c. H <sub>2</sub> O require 0.1 m./100 CaCl <sub>2</sub> ..	550
1.65 c.c. m./2 KCl in 100 c.c. H <sub>2</sub> O require 0.5 m./100 CaCl <sub>2</sub> ..	165
2.2 c.c. m./2 KCl in 100 c.c. H <sub>2</sub> O require 0.3 m./100 CaCl <sub>2</sub> ..	366
2.75 c.c. m./2 KCl in 100 c.c. H <sub>2</sub> O require 1.0 m./100 CaCl <sub>2</sub> ..	137.5
3.3 c.c. m./2 KCl in 100 c.c. H <sub>2</sub> O require 1.6 m./100 CaCl <sub>2</sub> ..	103

CaCl<sub>2</sub> acts upon KCl similarly as does NaCl, but it acts more powerfully; *i. e.*, the

coefficient of antagonization, KCl/CaCl<sub>2</sub>, is several hundred or a thousand times as great as that of KCl/NaCl, as Table V. shows.

The coefficients are not as regular as in the case of antagonization of KCl by NaCl. This is due to the fact that the minimal value of CaCl<sub>2</sub> at which it renders the KCl harmless can not be determined as sharply as the limit for NaCl. Why is less CaCl<sub>2</sub> required than NaCl? We can only answer with a suggestion first offered by T. B. Robertson, namely, that CaCl<sub>2</sub> produces its protective effect through the formation of a comparatively insoluble compound (in this case on the gills or the rest of the surface of the animal) while NaCl acts through the formation of a compound which is more soluble. This view is corroborated by the observation which we made, that Sr is just as effective to antagonize KCl as CaCl<sub>2</sub>, but that Mg is much less efficient. This would correspond with the well-known fact that many strontium salts are just as insoluble, if not more insoluble, than the calcium salts, while the magnesium salts are often incomparably more soluble, for instance in the case of the sulphates. BaCl<sub>2</sub> antagonizes KCl also powerfully, but, probably, in consequence of the fact that the substances formed at the surface of the animal or the gills, diffuse slowly into the cells, the fish do not remain alive as long if Ba is used as if the more harmless Ca and Sr are used.

It is very remarkable that CaCl<sub>2</sub> renders harmless any given concentration of KCl below 6.6 c.c. m./2 KCl in 100 c.c. of the solution, but not above this limit. This limit is exactly the same which we found in the case of antagonization of KCl by NaCl. Even the combination of NaCl and CaCl<sub>2</sub> does not permit us to render harmless more than 6.6 c.c. m./2 KCl in 100 c.c. of the solution.

If we try to render NaCl harmless by KCl and CaCl<sub>2</sub> we find that CaCl<sub>2</sub> can antagonize even a 6/8 m. and a 7/8 m. solution of NaCl, while KCl ceases to show any antagonistic effect if the NaCl solution exceeds m./2 or 5/8 m.

Experiments with pure CaCl<sub>2</sub> solutions give the result that this substance is harmless in a solution of that concentration in which this salt is contained in the sea-water. *Fundulus* can live indefinitely in a solution of 1.5 c.c. m./2 CaCl<sub>2</sub> in 100 c.c. Botanists have also found that weak solutions of CaCl<sub>2</sub> are comparatively little toxic. This gives us the impression that the effect upon the surface film of protoplasm produced by CaCl<sub>2</sub> is especially important for the protection of the protoplasm. This conclusion receives an indirect support by the well-known experiments of Herbst, who found that in sea-water deprived of calcium the segmentation cells of a sea-urchin embryo fall apart through the disintegration or liquefaction of a film which surrounds the embryo and keeps the cells together. If such eggs are brought back into solution containing calcium the film is restored and the cells come into close contact again.

It is therefore not impossible that the mechanism of the antagonism between KCl and NaCl is similar to that found between NaCl and ZnSO<sub>4</sub>. It seems only due to the high concentration of the NaCl in the sea-water and in the blood that, in addition to KCl and NaCl, CaCl<sub>2</sub> is needed. But the case is not so unequivocal as the previously mentioned cases of antagonism between only two electrolytes.

#### VIII

It is necessary for our understanding of the life-preserving action of salts that we do not depend merely on *conclusions* drawn from experiments, but that we must be able

to see directly in which way abnormal salt solutions cause the death of the cell. Such an opportunity is offered us through the observation of the eggs of the sea-urchin. If we put the fertilized eggs of the sea-urchin into an abnormal salt solution, a destruction of the cell gradually takes place. The destruction, as a rule, begins on the surface of the protoplasm, and consists very often in the formation and falling off of small granules or droplets. This process gradually continues from the periphery towards the center until the whole egg is disintegrated. For different salt solutions the picture of the disintegration is a little different, but sufficiently characteristic for a given solution, so that if one become familiar with these pictures, one is able to diagnose to some extent the nature of the solution from the way in which the cell disintegrates.

This process of disintegration can be observed if the eggs are put into a pure solution of sodium chloride or in a mixture of sodium chloride and calcium chloride, or in a mixture of sodium chloride and potassium chloride. If, however, all three salts are used in the proportion in which they occur in the sea-water no disintegration takes place and the surface of the egg remains perfectly smooth and normal. One gains the impression as if the protoplasm of the egg were held together by a continuous surface film of a definite texture. If we put the egg into an abnormal solution this surface film is modified and changed, and the change of the surface film is often followed by a gradual process of disintegration of the rest of the cell.

These observations on the sea-urchin egg, therefore, suggest the possibility that the combination of the three salts in their definite proportion and concentration has the function of forming a surface film of a definite structure or texture, around the

protoplasm of each cell, by which the protoplasm is kept together, protected against and separated from the surrounding media.

The previously mentioned observation of Herbst again shows the important rôle of calcium in this process.

### IX

The objection might be raised that the beneficial action of the three salts could only be proved on marine animals or on tissues of higher animals, which are said to be "adapted" to a mixture of NaCl, KCl and CaCl<sub>2</sub> in definite proportions. Experiments on fresh-water organisms, for which "adaptation" to a mixture of NaCl, KCl and CaCl<sub>2</sub> in these definite proportions can not be claimed, show that this objection is not valid. Ostwald worked with fresh-water crustaceans which he put into mixtures of various salts. It was found that these animals live longer in a mixture of NaCl + KCl + CaCl<sub>2</sub> than in a solution of NaCl, or NaCl + KCl, or NaCl + CaCl<sub>2</sub> of the same osmotic pressure.

Osterhout was able to show that the spores of a certain variety of *Vaucheria* die in a pure 3/32 m. solution of NaCl in 10 to 20 minutes, while they live in 100 c.c. 3/32 m. NaCl + 1 c.c. 3/32 CaCl<sub>2</sub> 2 to 4 weeks, and in 100 c.c. 3/32 m. NaCl + 1 c.c. 3/32 m. CaCl<sub>2</sub> + 2.2 c.c. 3/32 m. KCl 6 to 8 weeks. The reaction of the solution was strictly neutral and the NaCl the purest obtainable. The results remained the same after the NaCl had been recrystallized six times. Experiments with *Spirogyra* gave a similar result. The solutions were all 3/32 m. In NaCl the *Spirogyra* died in 18 hours; in NaCl + KCl in two days; in NaCl + KCl + CaCl<sub>2</sub> they lived 65 days. Osterhout caused wheat grains to develop in such solutions and measured the total length of the roots formed.

Nature of the Solution	Total Length of Roots after 40 Days
H <sub>2</sub> O .....	740 mm.
100 c.c. 3/25 NaCl .....	59 mm.
100 c.c. 3/25 NaCl + 2.0 3/25 CaCl <sub>2</sub>	254 mm.
100 c.c. 3/25 NaCl + 2.0 3/25 CaCl <sub>2</sub> + 2.2 3/25 m. KCl	324 mm.

These cases, to which many other similar observations might be added, prove that the life-preserving effect of the combination of NaCl + KCl + CaCl<sub>2</sub> in definite proportions is not due to the fact that organisms are "adapted" to this mixture but to a specific protective effect of the combination of the three salts upon the cells.

### X

It seems, therefore, to be a general fact that wherever tissues or animals require a medium of a comparatively high osmotic pressure—like our tissues—their life lasts much longer in a mixture of NaCl + KCl + CaCl<sub>2</sub> in the proportion in which these salts exist in the blood and in the ocean, than in any other osmotic solution, even a pure solution of NaCl. But the reader has noticed that there are considerable differences in the resistance of various organisms to abnormal solutions. While marine *Gammarus* die in half an hour in an isotonic solution of NaCl or cane sugar, red blood corpuscles or even the muscle of a frog can be kept for a day or longer in such a solution (of course even the muscle of a frog lives longer if the NaCl solution contains in addition KCl or CaCl<sub>2</sub>). What causes this difference?

Six years ago I found that the unfertilized eggs of the sea-urchin (*Strongylocentrotus purpuratus*) can keep alive and remain apparently intact in a pure neutral solution of CaCl<sub>2</sub> or of NaCl for several days at a temperature of 15°, while the fertilized eggs of the same female are killed in a pure neutral solution of CaCl<sub>2</sub> in a few hours. The same difference is

found for other salts also. What causes this difference? Several authors, Lillie, McClendon and Lyon, have suggested that it is due to the fact that the fertilized egg is more permeable to salts than the unfertilized egg. But the recent experiments by Warburg, which were confirmed and amplified by Harvey make it doubtful whether the salts which are not soluble in fats can enter the fertilized egg at all. I believe that the explanation of the difference is much more simple. The unfertilized egg is surrounded by a cortical layer and this layer is destroyed or modified in the process of fertilization. One result of this modification is the formation of the fertilization membrane, for which I have been able to show that it is readily permeable for salts. As long as the cortical layer of the unfertilized egg is intact, it prevents the surrounding salt solution from coming in contact with the protoplasm or at least it retards this process. If, however, the cortical layer is destroyed by fertilization the surrounding salt solution comes directly in contact with the protoplasm and if the solution is abnormal it can cause the disintegration of the surface layer of the protoplasm.

I am inclined to believe that differences in the resisting power of various cells or organisms to abnormal salt solutions are primarily due to differences in the constitution of the protective envelopes of the animals or the cells. Microorganisms which can live in strong organic acids or salt solutions of a high concentration probably possess a surface layer which shuts off their protoplasm from contact with the solution. For the protoplasm of muscle the rather tough sarcolemma forms not an absolute but nevertheless an effective wall against the surrounding solution.

But aside from differences of this kind there are other conditions which influence

the degree of resistance of cells to various solutions. I have found that the fertilized eggs of the sea-urchin will live longer in abnormal salt solutions if the oxidations in the egg are stopped, either by the withdrawal of oxygen or the addition of KCN or NaCN. Warburg and Meyerhof have drawn the conclusion that in a pure NaCl solution the rate of oxidations of the egg of *Strongylocentrotus* is increased and that it is this increase in the rate of oxidations which kills the eggs. But this increase of oxidations can not be observed in the eggs of *Arbacia* when they are put into a pure NaCl solution and, moreover, lack of oxygen prolongs the life of the fertilized egg just as well in solutions of NaCl + CaCl<sub>2</sub> or of NaCl + BaCl<sub>2</sub>, for which salts these authors do not claim that they can raise the rate of oxidations of the egg. I am inclined to believe that in the process during or preceding cell division, besides phenomena of streaming inside the cell, changes in the surface film of the protoplasm occur, whereby this film is more easily injured by the salts. If we suppress the oxidations we suppress also the processes leading to cell division and thereby retard the deleterious action of the abnormal salt solution upon the surface layer of the protoplasm of the egg.

## XI

If we now raise the question as to why salts are necessary for the preservation of the life of the cell we can point to a number of cases in which this answer seems clear. Each cell may be considered a chemical factory, in which the work can only go on in the proper way, if the diffusion of substances through the cell wall is restricted. This diffusion depends on the nature of the surface layer of the cell. Overton and others assume that this layer consists of a continuous membrane of fat

or lipoids. This assumption is not compatible with two facts, namely that water diffuses very rapidly into the cell, and second, that life depends upon an exchange of water-soluble and not of fat-soluble substances between the cells and the surrounding liquid. The above mentioned facts of the antagonism between acids and salts suggest the idea that the surface film of cells consists exclusively or essentially of certain proteins.

The experiments mentioned in this paper indicate that the rôle of salts in the preservation of life consists in the "tanning" effect which they have upon the surface films of the cells, whereby these films acquire those physical qualities of durability and comparative impermeability, without which the cell cannot exist.

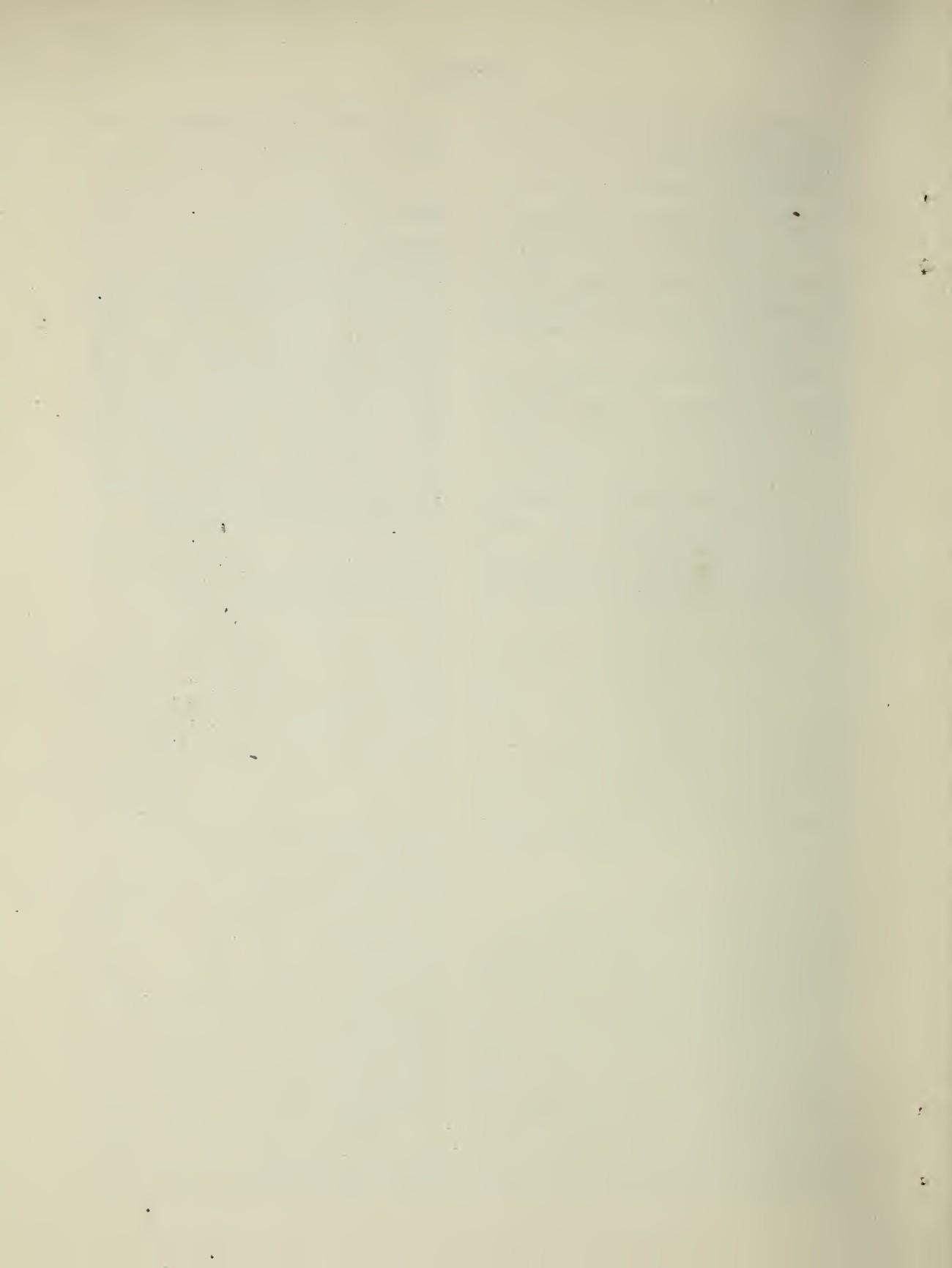
On this assumption we can understand that neutral salts should be necessary for

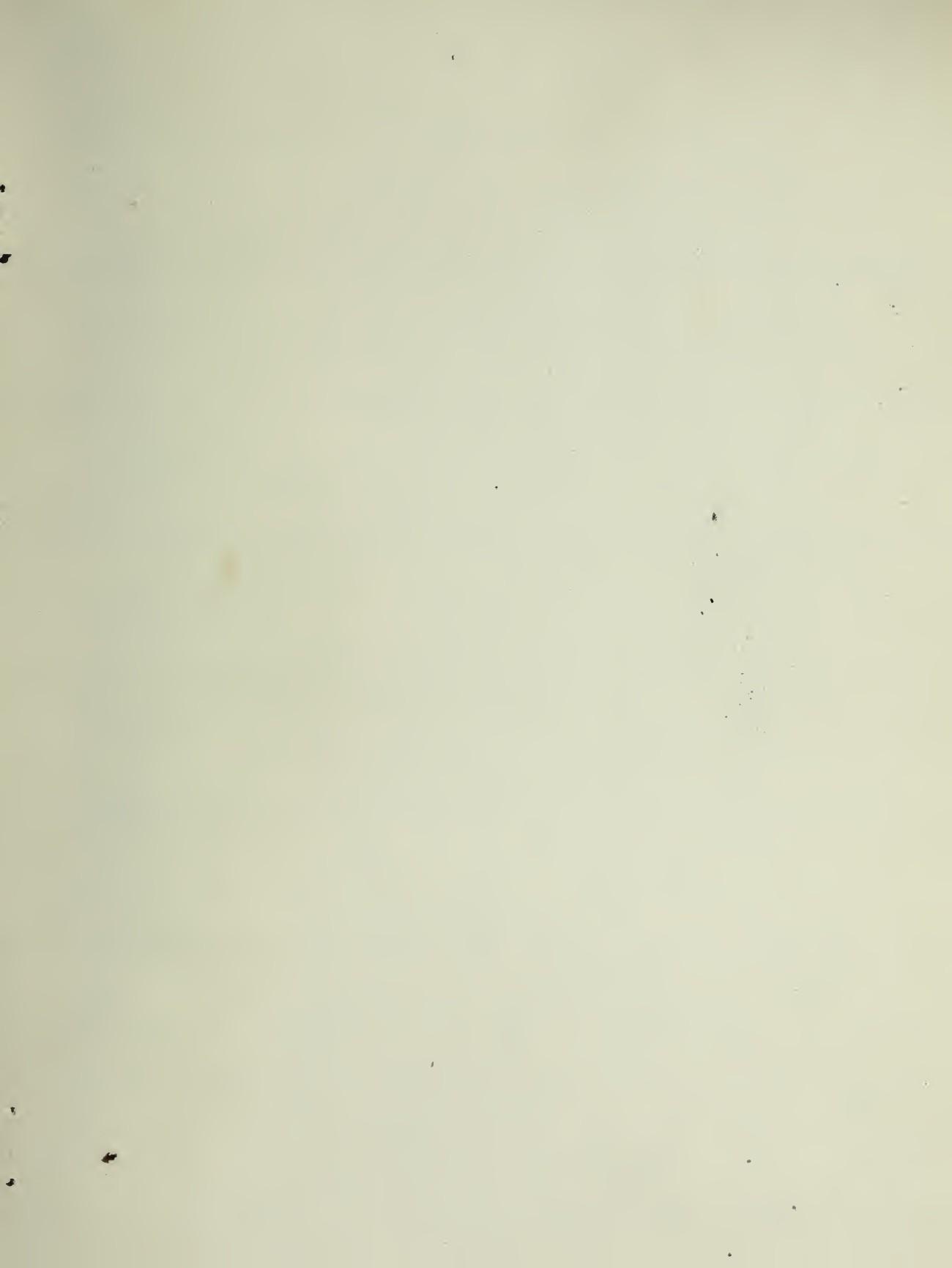
the preservation of life although they do not furnish energy.

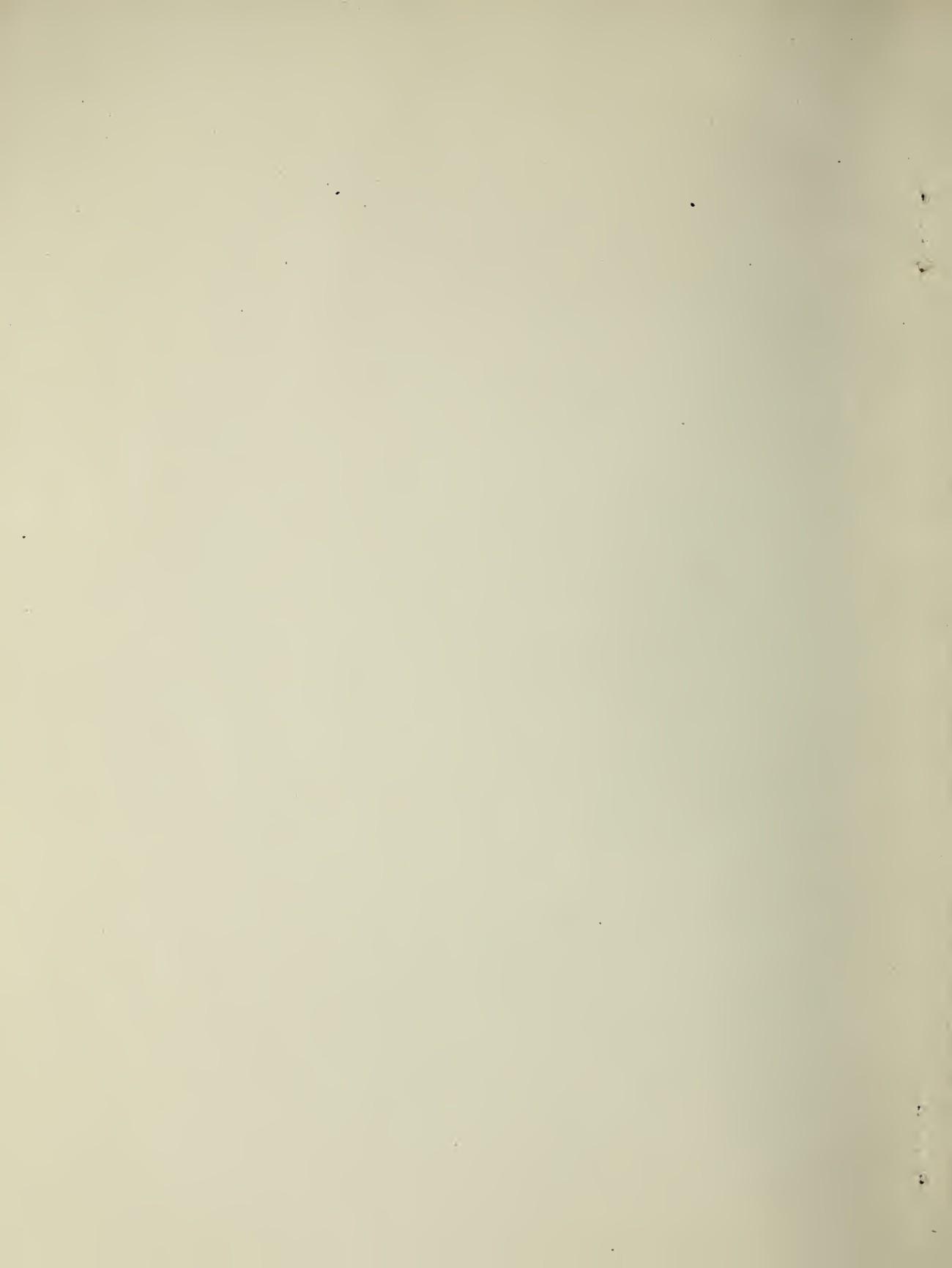
As far as the dynamical effects of salts are concerned it is not impossible that some of them belong also to the type of those mentioned in this paper. The fact that the addition of calcium to an NaCl solution prevents the twitchings of the muscle, which occur in the pure NaCl solution, suggests the possibility that the CaCl<sub>2</sub> merely prevents or retards the diffusion of NaCl through the sarcolemma. But other effects of salts, *e. g.*, the apparent dependence of contractility of the muscle upon the presence of NaCl; or the rôle of PO<sub>4</sub> in the nucleus, do not find their explanation in the facts discussed in this paper.

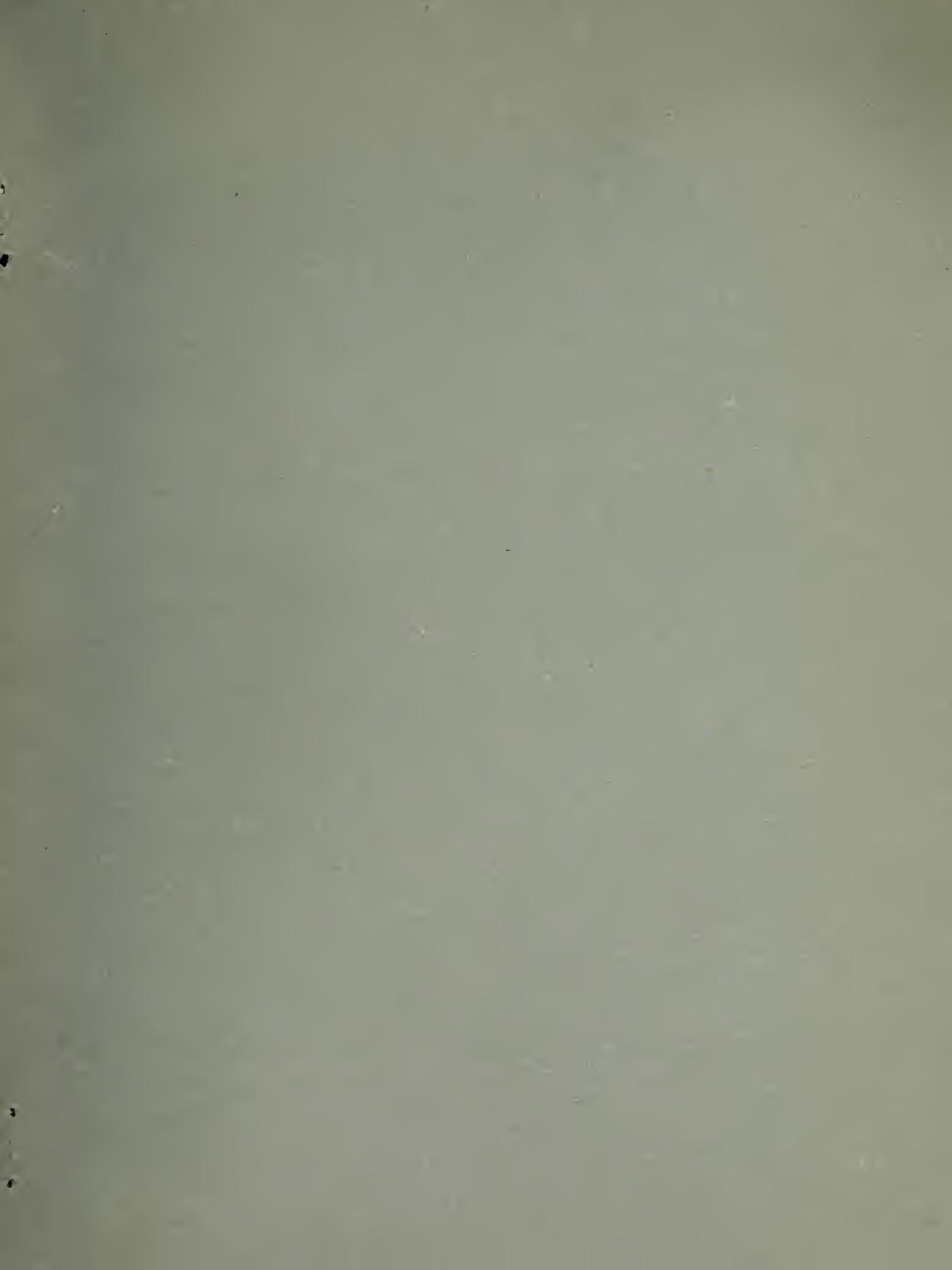
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